

Method of removing N-terminal alanine residues from polypeptides with *Aeromonas* aminopeptidase

Abstract

This invention describes a method of removing N-terminal alanine residues
5 from polypeptides, preferably recombinant proteins, using an aminopeptidase
derived from the marine bacterium *Aeromonas proteolytica*. Accordingly,
Aeromonas aminopeptidase (AAP; E.C. 3.4.11.10) can be used to remove N-
terminal alanyl residues from derivatives of human somatotropin (hST, human
10 growth hormone, or hGH), porcine somatotropin (pST), and bovine somatotropin
(bST), for example, to yield proteins having their native amino acid sequences. The
enzyme reactions can be carried out in free solution, or the AAP can be
immobilized on a solid support, for reactions carried out *in vitro*. An efficient
method for converting Ala-hGH to hGH, for example, comprises expression of Ala-
hGH in *E. coli*, recovery of inclusion bodies, solubilization and refolding in
15 detergent, detergent removal by ultrafiltration, selective precipitation, enzyme
cleavage, followed by two column chromatography steps.